Although we were unable to find mathematical correlation between the number of acentric fragments and dicentrics, it may be that their apperance reflects and elevated number of cells with more than one micronucleus. Tables 1 and 2 show that there is a good correlation in some cases between the total number of micronuclei and the frequency of acentric fragments after exposure to microwave radiation. In the case of VCM such correlation exists between the percentage of chromatid breaks and the total number of micronuclei.

Many chemicals are known to affect the spindle of the eukaryotic cell and thus have the capacity to induce aneuploidy. This was confirmed in our study, as the percentage of micronuclei in presons exposed to VCM was much higher than in those exposed to microwave radiation and who had the same frequency of aberrations.

Chromosome breaks, acentrics, dicentrics and ring chromosomes which fail to attach the normal or damaged spindle are predisposed to form micronuclei. With the micronucleus test itself it is impossible to determine whether some agent is aneuploidogen or clastogen. Recently, an immunochemical method which uses antikinetochore antibodies for detecting the content of micronuclei has been developed *(10)*. This technique enables differentiation between aneuploidogens and clastogens, and the micronucleus assay gets new dimensions. Eastwood and Tucker *(10)* have shown in their study that some chemical agents are aneuploidogens. In other words, a high percentage of micronuclei induced by chemicals contained whole chromosomes (kinetochore), while ionizing radiation was a potent clastogen inducing a very low percentage of micronuclei with a positively staining kineotchore.

The positive correlation between results of the micronucleus test and the chromosome aberration assay demonstrates that the two test are good indicators of exposure to genotoxic physical or chemical agents. In our experiment we are exposed both to radiation and chemical agents simultaneously. Future cytogenetic monitoring methods should be able to distinquish between different types of exposures, and finally, give a acliable image of the effects inducsd.

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USPOREDBA KROMOSOMSKIH ABERACIJA I MIKRONUKLEUSA U LJUDSKIM LIMFOCITIMA NAKON PROFESIONALNE IZLOŽENOSTI VINIL KLORID MONOMERU I MIKROVALNOM ZRAČENJU

SAŽETAK. *– Sličnosti i razlike mutagenog djelovanja mikrovalnog zračenja i vinil-klorid monomera proučavane su na limfocitima periferne krvi osoba profesionalno izloženih jednom od dva dva klastogena. Analize su izvedne na rezultatima mikronukleus-testa i strukturnih aberacija kromosoma. U radu je dokazano da se mutagena aktivnost mikrovalnog zračenja i vinil-klorid monomera može pratiti mikronukleus-testom i analizom strukturnih aberacija kromosoma. Vrijednosti i statističke signifikantnosti obaju testova usporedljive su. Razlike u djelovanju ta dva klastogena kvantitativne su i kvalitativne. Vinil-klorid monomer izaziva više oštećenja, u odnosu na mirkovalno zračenje i manji postotak oštećenja kromosomskog tipa (pojava dicentrika i ring kromosoma). Anafazni mostovi su u ovom uzorku kontinuirano prisutni dok se rijetko susreću u limfocitima ispitanika izložnih mikrovalnom zračenju. Postotak mikronukleusa kod istog postotka kromosomskih aberacija znatno je viši u osoba izloženih vinilkloridu monomeru u odnosu na izložene mikrovlanom zračenju. Ova studija upućuje na osnovne razlike u klastogenom djelovanju fizikalnog i kemijskog agensa.*

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Modification of Membrane Fluidity in Melanin-Containing Cells by Low-Level Microwave Radiation

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The treatment of a B16 melanoma cell line with 2.45-GHz pulsed microwaves (10 mW/cm^2, 10-μs pulses at 100 pps, 1-h exposure; SAR, 0.2 W/kg) resulted in changes of membrane ordering as measured by EPR (electron paramagnetic resonance) reporter techniques. The changes reflected a shift from a more fluid-like phase to a more solid (ordered) state of the cell membrane. Exposure of artificially prepared liposomes that were reconstituted with melanin produced similar results. In contrast, neither B16 melanoma cells treated with 5-Bromo-2-Deoxyuridine (3 μg/day × 7 days) to render them amelanotic, nor liposomes prepared without melanin, exhibited the microwave-facilitated increase of ordering. Inhibition of the ordering was achieved by the use of superoxide dismutase (SOD), which strongly implicates oxygen radicals as a cause of the membrane changes. The data indicate that a significant, specific alteration of cell-membrane ordering followed microwave exposure. This alteration was unique to melanotic membranes and was due, at least in part, to the generation of oxygen radicals.

Key words: microwaves, cell membrane, order, melanin, oxygen radicals

INTRODUCTION

The role of melanin in inducing cell-membrane alterations, particularly in response to non-ionizing radiation, has been a question of great interest. Melanin is a ubiquitous polymeric pigment that occurs in membrane-bound organelles or melanosomes of epidermal cells and several cell types in the eye. The major biological function of melanin is thought to be its role in photoprotection from harmful ultraviolet radiation, but melanin may also become a source of damage if its protective capabilities are overloaded [Pathak et al., 1976].

Melanin can function as a redox polymer, and it forms a paramagnetic species that is detectable by electron paramagnetic resonance (EPR). In addition to the per-

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sistent free radicals contained in melanin, transient free radicals are formed in melanin by a variety of chemical reactions, as well as by exposure to ultraviolet and visible light [Sarna and Sealy, 1984; Sealy et al., 1984]. The photo-reactions of melanin have been an area of particular interest with regard to interactions with oxygen, or in melanin's production and subsequent reaction with superoxide anion radicals [Sarna et al., 1980; Felix et al., 1978]. Using electron paramagnetic resonance spin-trapping techniques, Felix et al. [1978] demonstrated the capability of melanin to scavenge oxygen during photo-irradiation along with a further reduction of oxygen to hydrogen peroxide via a one-electron reduction that yielded superoxide anion. Korytowski et al. [1986] investigated the reaction of superoxide anion with melanin by spin-trapping techniques, and they demonstrated the formation of a transient, melanin, free-radical species. Studies have been performed to examine the interaction of melanin with electromagnetic radiation at various wavelengths: ultrasonic, visible, and ultraviolet [Sarna and Sealy, 1984; Sealy et al., 1984].

Little information has been gathered to examine the interaction between microwave radiation and melanin or other pigment-containing biological systems. In related studies, investigators have applied microwave energy to melanotic cells in the hope of inducing a thermal toxicity specific to tumor cells. These studies commonly employ B16 melanoma cell lines and evaluations of cell development [Santini et al., 1988], and antigenicity [Santini et al., 1986]. However, studies have not been performed to investigate possible interactions of microwave energy with the membrane bilayer of melanin-containing cells.

Studies of microwave effects on membranes of amelanotic cell lines and liposomes have become controversial. Liburdy and Vanek [1985] demonstrated that microwave radiation (2,450 MHz) reversibly increased membrane permeability to ^{22}Na in rabbit erythrocytes at a temperature that coincides with an apparent membrane phase-transition temperature. Several free-radical modulators were found to exert an influence on this effect [Liburdy and Vanek, 1985]. Similar studies in liposomes demonstrated a microwave-induced enhancement of permeability to ^{22}Na, at or near an apparent phase-transition temperature of the liposomal membrane [Liburdy and Magin, 1985]. Of interest is that addition of cholesterol to the liposomal membrane obliterated both the apparent phase-transition as well as the microwave induction of increased membrane permeability. Conversely, Rafferty and Knutson [1987] found no evidence of microwave-induced membrane alterations in liposomes of the same composition, but exposed at a different microwave frequency (0.93 GHz). Liu and Cleary [1988] further investigated the possibility of frequency-dependent alterations in liposome permeability. They demonstrated that differences between the results of Rafferty and Knutson [1987] and those of Liburdy and Magin [1985] were not due to differences in microwave frequency. They concluded that differences in protocols of microwave exposure may have accounted for the discrepancies between the studies. Further investigations must be performed to determine the factors that are pivotal in causing divergent results.

Our investigations centered on structural changes in membranes that are produced by low-level microwaves, which may be unique to melanin-containing cells, or to artificially prepared liposomes containing melanin. These membrane changes were assessed by EPR reporter techniques. With a series of EPR doxyl stearate probes, differing in the attachment site of the doxyl reporter group to the fatty acid (stearic acid), various locations within the membrane can be examined. The stearic acid acyl chain of the reporter orients itself in membranes parallel to the phospholipid fatty acids. Varying the position of the doxyl substituent (reporter group) on the fatty-acid chain allows for the examination of various depths of the membrane. Order parameters ($S_{||}$) calculated from the spectral characteristics of the reporters are an indication of membrane fluidity or order. Changes in membrane order have previously been correlated with alterations in protein function [Sinensky et al., 1979] and lipid peroxidation [Bruch and Thayor, 1983]. We report data that indicate a depth-dependent membrane interaction with microwave radiation that may be unique to melanotic cells, and that appears, in part, to be due to the generation of superoxide anions.

MATERIALS AND METHODS

Culture of B16 Melanoma Cell Line

The B16 cell line, line BL6 (DCT Tumor repository, Frederick, MD) originally isolated from C57BL/6 mice as described by Fidler [1973], was maintained as attached cells grown in culture. The melanoma cells were maintained on Minimal Essential Medium (MEM with Earles salts) containing 10% fetal bovine serum (MEM/10); at confluence they were passaged with 0.1% bovine pancreatic trypsin (Sigma) and maintained in T75 cell-culture flasks (Corning) as a cell culture with MEM/10 containing pen/strep under standard conditions (95% air, 5% CO_2, 37°C, 90% relative humidity). All tissue-culture-media reagents and PBS (phosphate buffered saline) were acquired from Gibco (Grand Island, NY), and serum was obtained from Sterile Systems (Logan, UT). Once grown to confluence or slightly subconfluent, melanoma cells were microwave- or sham-exposed (serum free). Following exposure, they were trypsin-quenched with fetal-calf serum, pelleted, washed with PBS (pH 7.4), and labeled with reporter agents as described in the EPR section below.

Treatment of B16 Cells with BrdU to Produce Amelanotic Cells

B16 cells were made amelanotic by adding 3 μg of 5-Bromo-2-Deoxyuridine (BrdU, Sigma Chemical Co., St. Louis, MO) per ml of medium per day for 7 days. Medium was changed on days 3, 5, and 7 after washing the cell layer. B16 cells shed melanin and BrdU prevents melanin production, resulting in amelanotic cells in 7 days [Wrathall et al., 1973]. The cells were then handled like non-BrdU-treated B16 cells for microwave exposures and EPR reporter techniques.

Liposome Preparation

Liposome vesicles reconstituted with melanin prepared from the persulfate oxidation of L-tyrosine (Sigma Chemical Co., St. Louis, MO) were formed by a modified procedure described by Van Rooijen and Van Nieuwmegen [1980]. A total of 100 mg of phosphatidylcholine was used. It was prepared from egg yolk (99% pure, Sigma Chemical Co., St. Louis, MO) and contained a variety of saturated and unsaturated acyl chains. To this phospholipid preparation 30% mol/mol cholesterol (Sigma Chemical Co.) was added. This mixture was suspended in 25 ml of chloroform/methanol 2:1 vol/vol and evaporated under vacuum in a 500-ml round-bottom flask on a rotary evaporator at 37°C for 2 hr. To this thin film, a 10-ml solution of melanin (1 mg/ml) in PBS (pH 7.4) was added and shaken vigorously for 15 min under nitrogen. The resulting suspension was sonicated under nitrogen for 1 min at

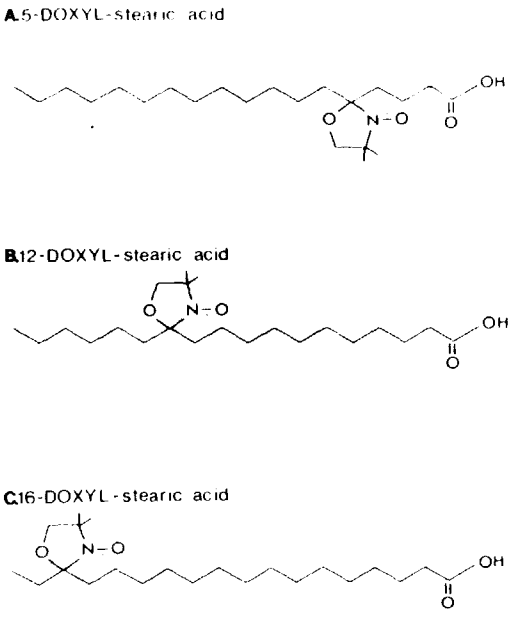

Fig. 1. Representative spin labels. **A:** 5-Doxyl stearic (5-DS) acid with paramagnetic nitroxide label on 5th carbon from carboxyl group (polar head group). **B:** 12-Doxyl stearic (12-DS) acid with nitroxide label on 12th carbon (middle of acyl chain) from carboxyl group. **C:** 16-Doxyl stearic (16-DS) acid with nitroxide label on 16th carbon (end of acyl chain) from carboxyl group.

4 °C by a Branson cell disruptor delivering 60 watts and equipped with a microprobe adapter. Remaining melanin not encapsulated inside the liposome was removed by centrifugation for 10 min at 1,000*g*. Empty liposomes (liposomes not containing melanin) were prepared identically with the exception of the melanin addition.

Spin Labeling and EPR Measurements

Manipulations of B16 melanoma cells and liposomes for EPR fluidity studies were similar. Liposomes and B16 cells were placed or grown in 5-ml MEM (serum free) in T-75 cell-culture flasks and arranged in a suitable configuration in the microwave chamber as described below. Following microwave exposure, liposomes were removed and the B16 cells were trypsinized for removal. To these membranes were added 30 μM of a doxyl stearic (DS) acid reporter with a nitroxyl-labeled paramagnetic moiety at the 5, 12, or 16 carbon on the stearic acid chain, labeling from the polar head-group region to the terminal end of the acyl chain (Fig. 1). Following incubation with the reporter (10 min at 37 °C), the liposomes or cells were washed with PBS 3 times to remove unbound label (11,000*g*, 10 min). The final pellet was transferred into a 25-μl micropipet, sealed, and placed into a quartz tube. The sample was supported vertically and placed in the cavity of a Bruker ER 200D-SRC EPR spectrometer, in a central field with a density of 3,350 G (335 mT), a

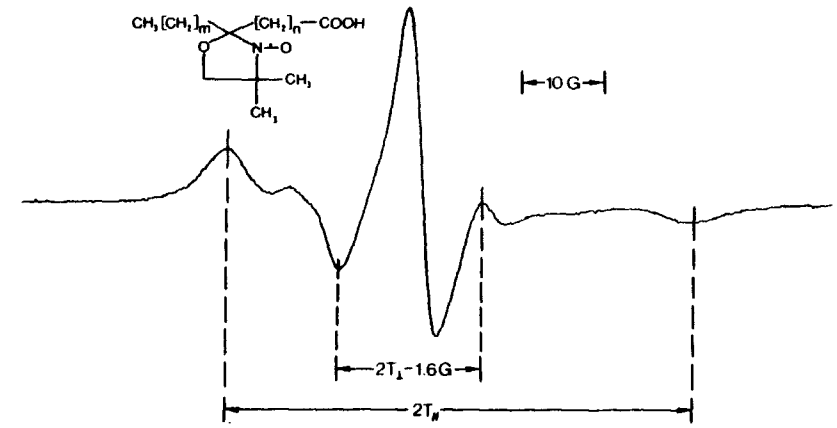

Fig. 2. Representative spectra of 5-doxyl stearic acid (5-DS) incorporated into a biological membrane. The figure demonstrates the spectral parameters $2T'_{\parallel}$ and $2T'_{\perp}$, which are used to calculate order parameters. In addition, the chemical structure of the doxyl stearic acids is shown with the following (M,N) values: 5-DS (12,3); 12-DS (5,10); and 16-DS (1,14).

sweep width of 100 G (10 mT), modulation frequency of 100 kHz, modulation amplitude of 2 Gpp (peak to peak), microwave power of 4.7 mWatts, time constant of 1.0 s, and a scan time of 1 ks. Order-parameter ($S_{\parallel}$ value) calculations were employed for the estimation of membrane fluidity. The spectral parameter $2T'_{\parallel}$ (Fig. 2) was employed to calculate $S_{\parallel}$ values. Computer-assisted signal averaging (IBM-PC with software (EPRDAS = 1.00 B/2.89) supplied by Adaptable Laboratory Software, Rochester, NY), curve smoothing, and amplifications were employed. The $S_{\parallel}$ values were calculated in accord with the technique of Sauerheber et al. [1977] and Gordon et al. [1983]. The order parameter calculations from the spectral characteristics are

$$S_{\parallel} = \frac{1}{2}\left[\frac{3(2T'_{\parallel} - T_{xx})}{(T_{zz} - T_{xx})} - 1\right].$$

T_{xx} and T_{zz} are splitting elements determined from host crystal studies. T_{zz} and T_{xx} splitting elements for 5- and 12-DS were taken from Seelig [1970]. Because T_{xx} and T_{zz} were not available for 16-DS, we utilized the splitting elements for 5-DS. Temperature was regulated by a nitrogen-controlled, variable temperature unit and spectra were obtained at 296 K.

Data presented are means of three or more samples for each group. Following spectral acquisition, order-parameter ($S_{\parallel}$) values were calculated according to the method described by Sauerheber et al. [1977]. $S_{\parallel}$ values allow for the expression of membrane fluidity independent of lateral phase separations and associated probe-probe interactions, which can be misconstrued as alterations in membrane fluidity. Briefly, values of $S_{\parallel}$ approaching unity indicate a completely hindered mobility (anisotropy) of the spin label in the membrane (Fig. 3e); in contrast, values approaching zero (isotropy) indicate the spin label tumbling freely in all dimensions in space (Fig. 3a). Those values between 0 and 1 (Fig. 3b–d) correspond to intermediate mobilities of the reporter molecules.

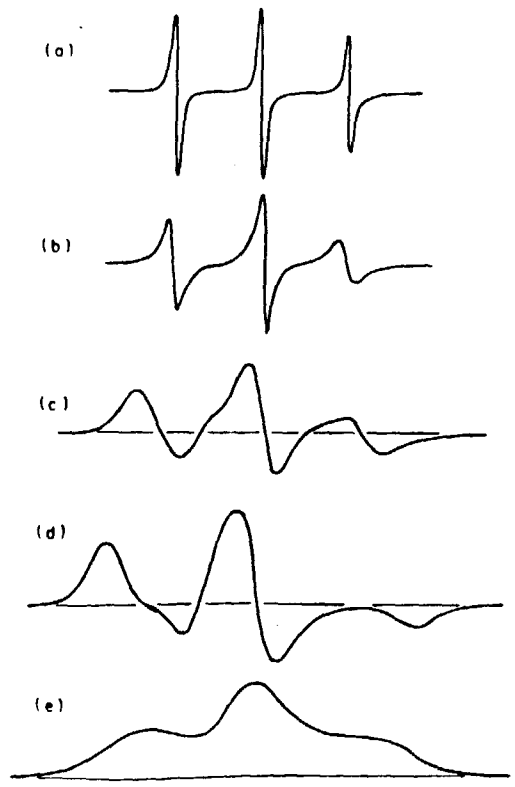

Fig. 3. Anisotopy of a nitroxide spin-label ESR spectrum under various conditions of motion. **a:** Isotropic spectrum from a nitroxide spin label randomly tumbling in a non-viscous solution. **b:** Lipid-dispersion spectrum from a nitroxide label undergoing anisotropic motion in a randomly oriented lipid dispersion. **c,d:** Lipid-dispersion spectra of a nitroxide label undergoing an increasingly hindered mobility. **e:** Powder spectrum from a nitroxide label randomly and rigidly oriented in a frozen solution (from Jones [1980]; printed with permission from Academic Press, Inc., New York).

Microwave Exposure Protocol

Cell-culture flasks were placed on a Styrofoam platform in an anechoic chamber lined with AN-77 and SPY-12 absorbing material. The flasks were positioned with the medium's surface perpendicular to the direction of energy propagation. Pulsed microwave energy (TE_{102}) at 2.45-GHz (10 μs at 100 pps) was generated by a Epsco PH40K generator and transmitted through a ferrite isolator (LS110LCI) and an HP-360D low-bandpass filter (> 30 dB at 2.45 GHz). The microwaves were transmitted by coaxial cable (RG-214/U) through a bidirectional coupler (Narda-30V) that provided measures of forward and reflective power. Final coupling of microwaves was through a custom-designed horn antenna (Narda). The Sytrofoam platform containing the cell flask was positioned beyond the $2d^2/\lambda$-defined, far-field boundary.

The power-density of incident microwaves (10 mW/cm^2, rms) at the cell culture's media surface was determined by a calibrated, 2.45-GHz Narda 8201 electromagnetic monitor. A specific absorption rate (SAR) of 0.2 W/kg was calculated for the power density of 10 mW/cm^2 based on temperature measurements during microwave exposure with a non-perturbing Fluoroptic (1000B system) thermal probe [Durney et al., 1980].

Control (sham) culture flasks were placed in the anechoic chamber with the irradiated flasks during the 1-h exposure. The control flasks, however, were shielded from the microwave field by AN-77 microwave absorbing material. To exclude the possibility of a temperature-only effect, several 4-h exposures were conducted in which a fluoroptic 1000B temperature measurement system was used to monitor both the exposed and non-exposed cell flasks, as well as the internal temperature of the anechoic chamber. The difference between exposed and non-exposed flasks was within ± 0.2 °C during exposures. Although sham-exposed flasks examined with identical EPR parameters were used as controls, it should be noted that magnetic-field interactions from the EPR spectrometer cannot fully be ruled out as a component in these experimental results. Studies that permit comparison of results from EPR probes and fluorescent probes could be performed to rule out possible magnetic/microwave interactions. One drawback in the use of fluorescent probes for membrane-order determinations is the lack of depth-dependent probes, thus eliminating the evaluation of regional membrane alterations with these probes.

STATISTICS

Group comparisons conducted by Student's t-test or ANOVA, where significant differences were obtained for $P < .05$ [Steel and Torrie, 1980].

RESULTS

Figure 4 shows that microwave irradiation of B16 melanoma cells significantly altered membrane characteristics reported by two of the three reporters that were used to measure membrane fluidity. The 5-doxyl stearic acid probe, which sampled the polar head-group region, reported a 5–10% increase in membrane order following microwave radiation that was 90% inhibitable by the addition of superoxide dismutase (SOD, 1,500 units/ml). Similarly, the 12-doxyl stearic acid reporter (sampling the middle-membrane region) reported a dramatic increase (> 20%) in membrane order following microwave exposure over the order of sham-exposed cells (Table 1). The application of SOD (1,500 units/ml) was unable to completely inhibit (~ 15%) the alteration in membrane order produced by microwave radiation. The inability of SOD to inhibit the membrane changes reported by 12-doxyl stearic acid may be related to the difficulty of SOD to cross biological membranes. If both extracellular and intercellular free radicals are being formed, then SOD will inhibit only the extracellular component. Of interest was the apparent inability of microwave radiation to alter membrane fluidity characteristics reported by 16-DS. This may be secondary to the relative absence of double bonds on acyl chains in this region, or a function of the high mobility of the acyl chains at this depth in the bilaminar leaflet.

Figure 5 demonstrates that B16 cells treated with 5-bromo-2-deoxyuridine to produce amelanotic cells showed results that contrast dramatically with those found

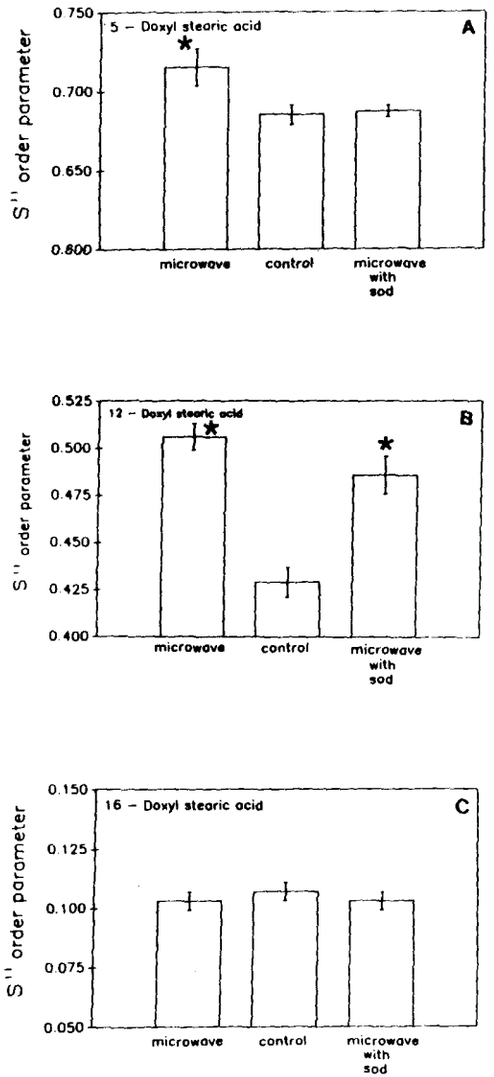

Fig. 4. The effect of microwave radiation [2.45 GHz, 10 mW/cm^2 (10-μs pulses, 100 pps), 1 h exposure] on membrane fluidity characteristics of B16 melanoma cells in the presence and absence of superoxide dismutase (SOD, 1,500 units/ml). **A:** Membrane fluidity reported by 5-DS, near the polar head-group region. **B:** Membrane fluidity reported by 12-DS, near the middle of the lipid acyl chains. **C:** Membrane fluidity reported by 16-DS, near the terminal region of the acyl chain. (*$P < .05$, ANOVA.)

TABLE 1. Summary of Order Parameters ($S_{||}$) Following B16 Melanoma Microwave Irradiation [2.45 GHz, 10 mW/cm^2 (10-μs pulses, 100 pps); 1-h exposure]

Reporter	Sham exposed (N)	10 mW/cm^2 × 1 h (N)	10 mW/cm^2 × 1 h + SOD (N)
B16 melanoma cells—no BrdU treatment			
5-doxyl stearate (5-DS)	.6839 ± .0063 (4)	.7153 ± .0118 (4)*	.6871 ± 0.037 (4)
12-doxyl stearate (12-DS)	.4295 ± .0041 (4)	.4969 ± .0058 (4)*	.4858 ± .0145 (3)*
16-doxyl stearate (16-DS)	.1032 ± .0038 (3)	.1072 ± .0038 (3)	.1033 ± .0038 (3)
B16 melanoma cells treated with 5-bromo-2-deoxyuridine (BrdU), 3 μg/day × 7 days to produce amelanotic cells			
5-doxyl stearate (5-DS)	.6841 ± .0044 (5)	.6643 ± .0062 (4)	
12-doxyl stearate (12-DS)	.4214 ± .0242 (3)	.3932 ± .0244 (3)	
16-doxyl stearate (16-DS)	.0996 ± .0002 (3)	.1033 ± .0038 (3)	

*$P < .05$, compared to sham (ANOVA).

in melanotic cells. Microwave exposure [2.45 GHz, 10 mW/cm^2 (10-μs pulses, 100 pps), 1 h] caused a slight but insignificant decrease in order as reported by 5-DS and 12-DS. The observed effect indicates a mechanism different from that seen in the melanotic B16 cells (Table 1).

Figure 6 (Table 2) shows the effect of microwave irradiation (2.45 GHz, 10 mW/cm^2 (10-μs pulses, 100 pps), 1-h exposure) on artificial liposomal membranes reconstituted with melanin. Similar changes in membrane order were seen in this model as in the untreated B16 melanoma cells, with the exception of the 16-DS reporter. Both 5-DS and 12-DS reported an increase in membrane order following microwave exposure, with 12-DS showing the more significant effect. Conversely, the 16-DS reporter showed a small fluidization in the terminal end of the acyl chain. It is possible that peroxidation of the unsaturated bonds on the acyl chain located in the 5- and 12-DS region may provide an increase in rotational movement further down the acyl chain as reported by 16-DS.

Figure 7 (Table 2) presents the results on liposomal systems lacking melanin reconstitution. A small, fluidizing effect in the 5- and 12-DS reporter regions occurred, but no effect was seen in the 16-DS region of the amelanotic liposome membrane. As in the amelanotic B16 cell line, it appears that the membrane changes produced by microwave exposure in the amelanotic liposomes were different from those produced in melanotic liposomes.

DISCUSSION

The results presented in this paper demonstrate the existence of a unique, field-induced alteration of membrane order associated with melanotic cells and with artificial membranes reconstituted with melanin. The membrane effects appeared to involve an interaction between relatively weak microwave fields and melanin. As we noted earlier, an interaction between the intense magnetic field produced by the EPR spectrometer and the melanotic end point cannot be ruled out as a factor in our experimental results. The mechanism(s) associated with these membrane alterations

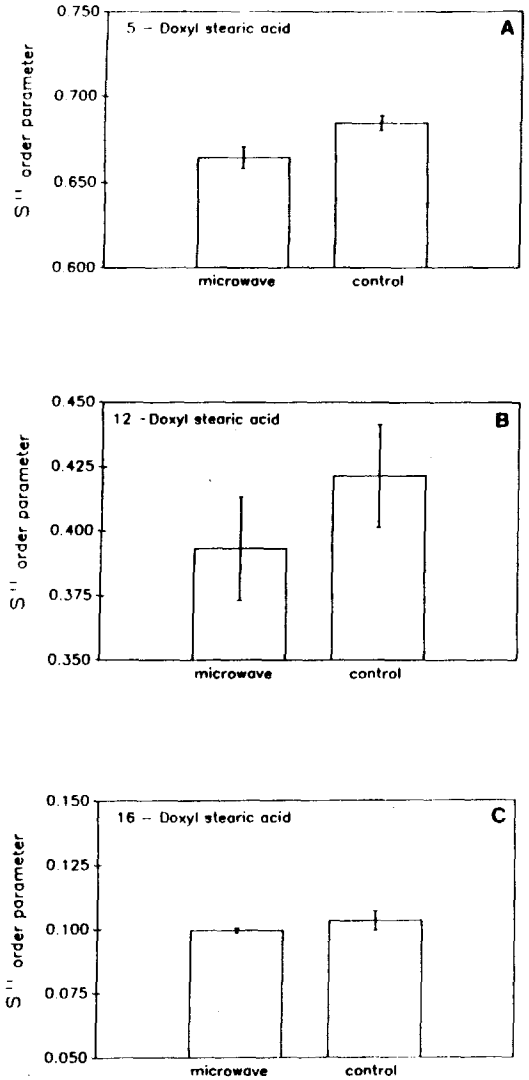

Fig. 5. The effect of the microwave radiation on membrane-fluidity characteristics of B16 melanoma cells treated with 5-bromo-2-deoxyuridine (BrdU), 3 μg/day × 7 days to produce amelanotic cells. **A:** Membrane fluidity reported by 5-DS near the polar head group region. **B:** Membrane fluidity reported by 12-DS near the middle of the lipid acyl chains. **C:** Membrane fluidity reported by 16-DS, near the terminal region of the acyl chain. (*$P < .05$, ANOVA.)

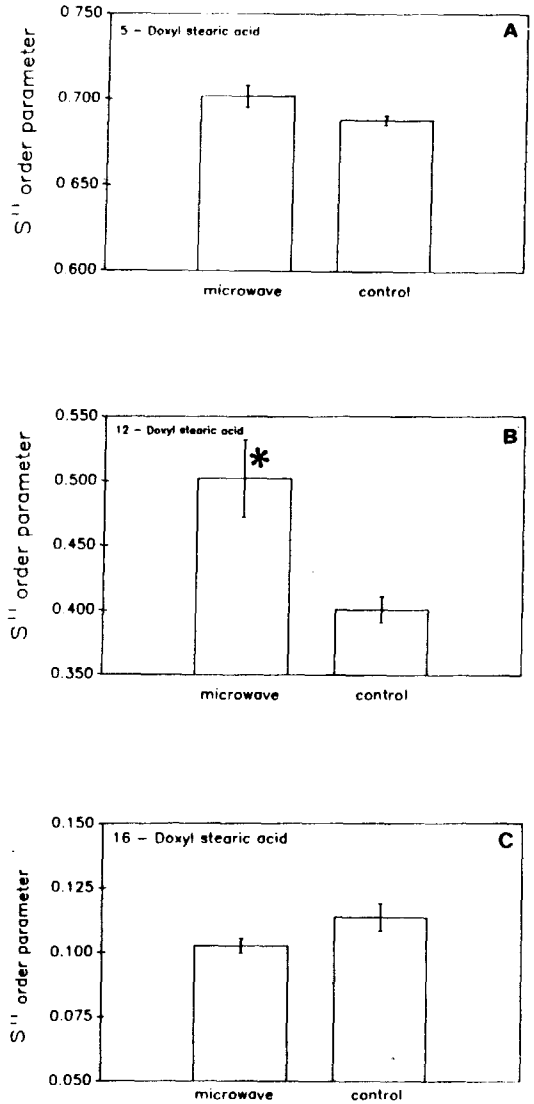

Fig. 6. The effect of the microwave radiation on membrane-fluidity characteristics of phosphatidylcholine/cholesterol liposomes reconstituted *with* melanin. **A:** Membrane fluidity reported by 5-DS, near the polar head-group region. **B:** Membrane fluidity, reported by 12-DS, near the middle of the lipid acyl chains. **C:** Membrane fluidity reported by 16-DS, near the terminal region of the acyl chains. (*$P < .05$, ANOVA.)

TABLE 2. Summary of Order Parameter ($S_{\parallel}$) Following Microwave Irradiation [2.45 GHz, 10 mW/cm² (10-μs pulses, 100 pps), 1-h exposure] of Egg Lecithin Liposomes With or Without Reconstituted Melanin (1 mg/ml)

Egg lecithin liposomes reconstituted with melanin (1 mg/ml)		
Reporter	Sham exposed (N)	10 mW/cm² × 1 h (N)
5-doxyl stearate (5-DS)	.6878 ± .0028 (4)	.7018 ± .0064 (4)
12-doxyl stearate (12-DS)	.4008 ± .0103 (3)	.5020 ± .0426 (3)*
16-doxyl stearate (16-DS)	.1025 ± .0028 (4)	.1137 ± .0053 (4)
Egg lecithin liposomes—no melanin		
Reporter	Sham exposed (N)	10 mW/cm² × 1 h (N)
5-doxyl stearate (5-DS)	.6713 ± .0027 (3)	.6559 ± .0098 (3)
12-doxyl stearate (12-DS)	.4035 ± .0060 (3)	.3825 ± .0088 (3)
16-doxyl stearate (16-DS)	.1054 ± .0056 (3)	.1067 ± .0043 (3)

*$P < .05$, compared to sham (ANOVA).

is (are) unclear. The evidence indicates the involvement of oxygen radicals, because superoxide dismutase inhibited field-induced effects. Treatment of the B16 melanoma cell line with an agent shown previously to produce amelanotic cells [Wrathall et al., 1973] completely abolished the effects seen in the B16 melanotic cell line, which strongly supports the involvement of melanin in these reactions. Artificial liposomes reconstituted with melanin showed similar alterations in bilayer structure following microwave exposure, but these effects were not duplicated in liposomes devoid of melanin.

The photochemistry and photobiology of melanin in relation to its involvement with free radicals have been extensively studied. Several investigators have related melanin to free-radical production [Felix et al., 1978, 1979], as well as to its ability to act as a free-radical scavenger [Goodchild et al., 1981]. Evidence has been obtained to demonstrate the production of superoxide during ultraviolet radiation of melanin systems [Persad et al., 1983], as well as documenting melanin free-radical scavenging properties [Korytowski et al., 1986]. Reactive oxygen species formed from the interaction of melanin systems with radiation may subsequently produce membrane alterations via lipid peroxidation [Kirsch et al., 1987; Bruch and Thayor, 1983; Phelan and Lange, 1991]. In our system, microwave radiation has been shown to produce an ordering effect in membranes of melanin-containing cells or liposomes. Inhibition of these ordering effects by superoxide (SOD) in the melanotic membranes following microwave exposure strongly indicates the involvement of oxygen-free radicals in these reactions. Oxygen-free radicals have been implicated in membrane lipid-peroxidation reactions associated with membrane ordering [Bruch and Thayor, 1983; Phelan and Lange, 1991]. The superoxide scavenger (SOD) has also been shown to inhibit the formation of lipid peroxidation product malondialdehyde (MDA) following ischemia–reperfusion reactions [Kirsch et al., 1987].

The membrane alterations seen in our melanin systems are similar to those found by Bruch and Thayor [1983] in non-melanin liposomal systems undergoing lipid-peroxidation reactions. Using similar EPR reporting techniques, Bruch and Thayor [1983] demonstrated that the region reported by 12-doxyl stearic acid (12-DS) was uniquely sensitive to lipid peroxidation reactions, most likely due to the abun-

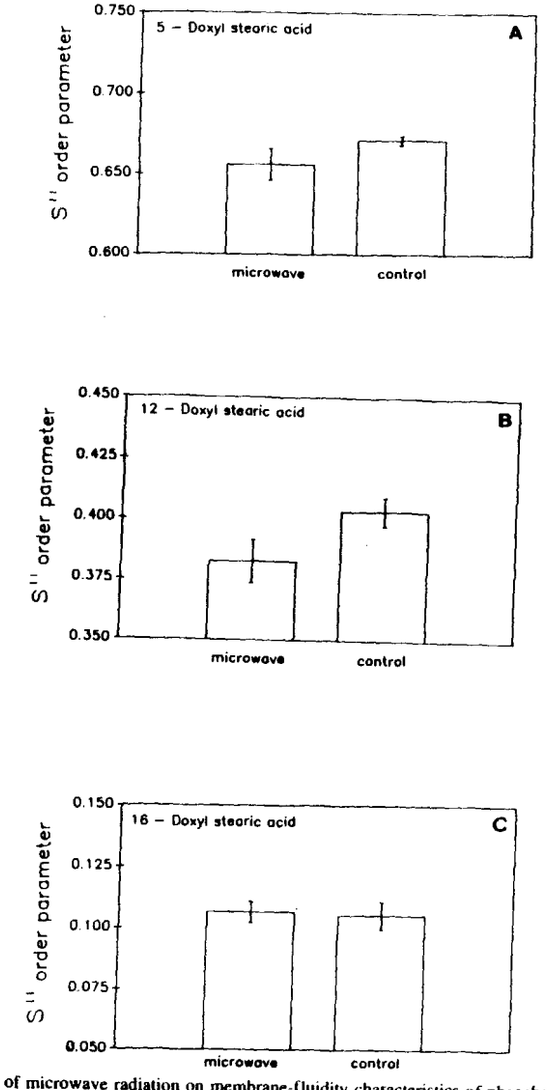

Fig. 7. The effect of microwave radiation on membrane-fluidity characteristics of phosphatidylcholine/cholesterol liposomes reconstituted *without* melanin. **A:** Membrane-fluidity characteristics of phosphatidylcholine/fluidity reported by 5-DS, near the head-group region. **B:** Membrane fluidity reported by 12-DS, near the middle of the lipid-acyl chains. **C:** Membrane fluidity reported by 16-DS, near the terminal region of the acyl chains. (*$P < .05$, ANOVA.)

dance of unsaturation in that area. Our results demonstrate a 25% increase in order in the region reported by 12-DS that may be related to lipid peroxidation. The increase in order found in this region correlated well with the degree of peroxidation found in Bruch's system. Further studies need to be performed to directly relate microwave-induced membrane ordering to lipid peroxidation in melanin-containing systems. This degree of membrane ordering in the 12-DS region is similar during active peroxidation, in liposomes producing between 15 and 20 nMole of malondialdehyde/mg lipid [Bruch and Thayor, 1983]. Changes in membrane order have been shown to alter protein function of integral proteins such as ATPase [Sinensky et al., 1979]. Thus, it is clear that the degree of membrane ordering (rigidification) produced can significantly affect biological systems.

The mechanisms by which melanin produces phototoxic reactions *in vivo* are still not well understood. Future studies need to be performed to determine the involvement of melanin's intermediates and metabolites in these reactions. Several biologically active derivatives and intermediates are formed during the biosynthesis of melanin [Koch and Chedekel, 1987; Graham et al., 1978]. Melanin contained in the subcellular melanosome of melanocytes is only one possible reactant in a series of precursors and metabolites found during melanogenesis. The autoxidation and photolysis of catechols during melanogenesis produce a number of highly reactive, free-radical species that have been demonstrated to form other free-radical species, including superoxide anion, hydroxyl radicals (via Harber Weiss or Fenton reactions), as well as semiquionones and indolyl nitrogen-based radicals [Chedekel et al., 1984; Kalyanaramah et al., 1982]. These intermediates and metabolites are shed actively by these cells, and many of them have been documented as photochemically unstable and products of free radicals [Koch and Chedekel, 1987; Rorsman et al., 1973]. The oxygen-free radicals produced by photolysis have been demonstrated to initiate lipid peroxidation, DNA strand breakage, and protein damage [Kirsch et al., 1987; Bruch and Thayor, 1983; Mason, 1979; Miranda et al., 1984].

Our data demonstrate the ability of microwave radiation to uniquely interact with melanin-containing cells and to produce membrane-order changes associated with the production of free radicals. There are several reaction schemes that could be hypothesized to account for these observations. Although melanin has previously been shown to scavenge free radicals, microwave interaction with this polymer may change its structure or excited state in a way that allows it to combine with oxygen to produce superoxide radicals. Alternatively, microwaves may directly or indirectly reduce oxygen to produce superoxide, which may interact with melanin to produce the membrane alterations seen. Membrane order can have biological implications with regard to alterations in protein function and permeability characteristics associated with normal physiological function. Work is currently in progress to evaluate protein alterations associated with these changes, in addition to extending these studies to *in vivo* evaluations of microwave interaction with highly melanotic tissues (i.e., iris, retina, and pigment epithelium). Future studies will involve investigations to determine what rôle these various components play in microwave-induced alterations of melanotic cell membranes, as well as in surrounding amelanotic tissue. These studies may aid in the explanation of changes observed in other membranes. Our findings support theories purporting the involvement of melanin in radiation-induced, free-radical formation, but further extend this theory to energies found in the low-level range of microwave energy.

ACKNOWLEDGMENTS

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Calcium Binding to Metallochromic Dyes and Calmodulin in the Presence of Combined, AC-DC Magnetic Fields

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The possibility that weak, ac and dc magnetic fields in combination may affect binding equilibria of calcium-ions (Ca^{2+}) was investigated with two metallochromic dyes as calcium-binding molecules: murexide and arsenazo III. Calcium-dye equilibria were followed by measuring solution absorbances with a fiber-optic spectrophotometer. A Ca^{2+}-arsenazo solution was also used indirectly to monitor the binding of Ca^{2+} to calmodulin. Parallel, ac and dc magnetic fields were applied to each preparation. The ac magnetic field was held constant during each of a series of experiments at a frequency in the range between 50 and 120 Hz (sine wave) or at 50 pps (square wave) and at an rms flux density in the range between 65 and 156 μT. The dc magnetic field was then varied from 0 to 299 μT at 1.3 μT increments. The magnetic fields did not measurably affect equilibria in the binding of metallochromic dyes or calmodulin to Ca^{2+}.

Key words: murexide, arsenazo III, absorbance, cyclotron resonance, ELF

INTRODUCTION

A number of observations has been reported of a resonant response of biological preparations to combined application of ac and dc magnetic fields. For example, McLeod and Liboff [1986] showed that the motility of diatoms on a calcium-impregnated agar substrate was highly dependent on calcium-ion concentration. When they exposed the diatoms to a combination of parallel ac and dc magnetic fields they found that the motility of the diatoms exhibited a marked reaction, which was centered about the cyclotron-resonance frequency of the unhydrated calcium ion. The resonant frequency was directly proportional to the strength of the dc magnetic field. These experiments are strong evidence of a biological response that is clearly related to resonant, ac-dc, magnetic-field conditions.

Liboff et al. [1987] exposed human lymphocytes suspended in a solution with

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Resonance Effect of Microwaves on the Genome Conformational State of *E. coli* Cells

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Cellular Biology, Microwave Bioaction, Radiation Damage, Repair

The effect of low intensity microwaves on the conformational state of the genome of X-irradiated *E. coli* cells was studied by the method of viscosity anomalous time dependencies. It has been established that within the ranges of 51.62–51.84 GHz and 41.25–41.50 GHz the frequency dependence of the observed effect has a resonance nature with a resonance half-width of the order of 100 MHz. The power dependence of the microwave effect within the range of 0.1–200 μW/cm² has shown that a power density of 1 μW/cm² is sufficient to suppress radiation-induced repair of the genome conformational state. The effect of microwave suppression of repair is well reproduced and does not depend on the sequence of cell exposure to X-rays and microwave radiation in the millimeter band. The results obtained indicate the role of the cell genome in the resonant interaction of cells with low intensity millimeter waves.

Introduction

At present a significant body of evidence has been collected on the ability of microwaves in the millimeter range to bring about biological effects including those on the cellular level [1, 2]. It has been found that microwaves can influence the processes of gene expression [3–5]. The specific features of such interaction are dependence on frequency and also effectiveness of low intensity microwave radiation which does not result in significant heating of the irradiated object. One of the possible explanations of these facts accounts for the influence of millimeter waves on the genome conformational state [6]. The genome conformational state (GCS) is expressed as the space-topological organization of the entire chromosomal DNA, which is ensured, among other things, by the supercoiling of DNA and DNA protein bonds. The GCS changes play a significant role in all elementary genetic processes – transcription, replication, repair.

The hypothesis which accounts for the influence of millimeter radiation most evident in the case of stressed systems [1, 7] among them bioobjects subjected to ionizing radiation [6] has repeatedly been verified.

The influence of millimeter waves on the process of the GCS repair after *E. coli* K 12 cell exposure to X-rays was examined in this work. As a test for appearance and repair of changes in a chromosomal DNA we used the method of the anomalous viscosity time dependencies (AVTD) in cell lysates [6].

Materials and Methods

Microwave and X-ray irradiation

A block diagram of the experimental unit used for microwave irradiation of cell suspension is given in Fig. 1. A G4-141 generator served as the source of extremely high frequency electromagnetic radiation (EHF EMR). In the course of irradia-

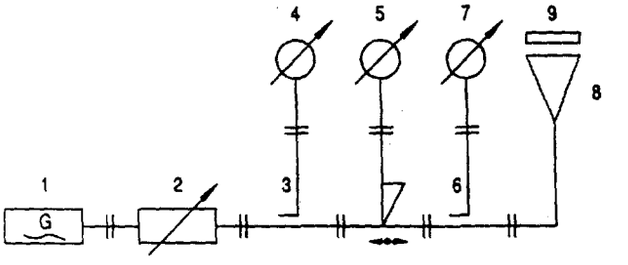

Fig. 1. Block diagram for microwave irradiation of cell suspension: 1 – EHF EMR generator; 2 – controlled attenuator; 3, 6 – directional coupler; 4 – frequency analyzer; 5 – measurement line (VSWR-meter); 7 – power meter; 8 – pyramidal horn; 9 – cell suspension.

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tion the frequency, the output power, as well as the voltage standing wave ratio (VSWR) were controllable. Frequency instability was 1 MHz, error in the measurement of the output power did not exceed 10% and the value of VSWR in the waveguide was not more than 1.6. Irradiation of a cell suspension (1.5 mm thickness) was carried out in Petri dishes, 50 mm in diameter, by means of a pyramidal horn having dimensions 40 × 50 mm^2.

The space distribution of the power density (PD) on the surface of the suspension was measured by means of a dipole EHF probe [8]. With the irradiation frequencies used the local PD values at the surface of the suspension differed by nearly an order of magnitude. But frequency changes of ± 200 MHz did not lead to significant changes of the pattern of PD distribution. At the same time frequency changes in a wide range (of the order of units GHz) could lead to a marked displacement of PD minima and maxima up to their inversion. In the event of parity of output power in the waveguide, the PD value, averaged over the whole surface under irradiation, did not change.

The specific absorption rate (SAR) was measured in two ways: by the acoustic method [9] and the calorimetric method. The suspension temperature was measured by a microthermocouple.

Cells were subjected to X-rays (XR) using a radiological unit RUP-150. The distance from the focus to the suspension was 40 cm, average radiation energy – 50 keV, dose rate 0.7 Gy/min. Microwave and X-irradiation of cells was carried out at ambient temperature.

Preparation of bacterial cells for experiments and cell lysis

The following strains were used in the work: *E. coli* K12: AB1157 F^- thr-I ara-14 leu-B6 proA2 lacGI tsx-33 supE44 galK2 hisG4 rfbDI mgl-51 rpsL31 xyl-5 mtl-I argE3 thi-I λ^- rac^-; G62 F^+ proA23 lac-28 trp-30 his-51 rpsLR and also strain RM117 which is isogenic with strain AB1157. Cells were cultivated by standard methods in Luria broth or minimal medium M-9 [10]. The *E. coli* cultures used in the experiments were kept in spreadings on the Hottinger nutrient agar at 3–4 °C.

Before irradiation, cells from the night culture were resuspended in concentrations of $3 \div 9 \times 10^7$ cells/ml in a salt buffer M-9. Cells were kept under these conditions for 1 h before irradiation.

After irradiation, cells were lysated by gradually adding LET-lysozyme (LET-medium: 0.5 M Na_2 EDTA, 0.01 M Tris-HCl, pH 7) in a concentration of 3 mg/ml, LET-sarcosyl (2%) and LET-papain (3 mg/ml) with 10 to 15 min intervals between addition of each agent. 0.3 ml LET-lysozyme, 1.0 ml LET-sarcosyl, 0.7 ml LET-papain were added to 1 ml of cell suspension. The lysates were then kept in darkness at a temperature of 30 °C for 40 h, after that the AVTD were measured.

Method of anomalous viscosity time dependencies

This method is based on the fact that in solutions of high-polymer DNA, placed in a rotary viscosimeter, radial migration of DNA, which is a directed movement of macromolecules towards the inner cylinder of the viscosimeter (rotor), is observed [11].

Measurements were carried out in a rotary cylindrical viscosimeter with an automatic record of the rotor's rotation period [6]. In the unit used, the rotor was set in motion by a constant moment of force created by an external electromagnetic field.

Upon completion of the lysis the rotor was suspended on the meniscus of the lysate examined. Thereafter the lysate was placed in a thermostatically controlled (30 °C) jacket of the viscosimeter for measurement.

When the external electromagnetic field is switched on, the rotor starts moving. In the initial stage of measuring the rotor's rotation period (T), the lysate viscosity increases due to a radial migration of macromolecules. This results in an increased rotation period of the rotor since the period is proportional to the specific viscosity (Fig. 2, curve 1). After the DNA macromolecules had deposited on the external surface of the rotor the velocity of its rotation decreased to the value typical of a pure solvent. The dependence of the rotor's rotation period in the cell lysate on the time after the rotor's rotation starts (t) is called anomalous viscosity time dependence.

It should be noted here that AVTD cannot be observed in protein solutions, because radial migration doesn't take place in solutions of molecules with weights less than 10^6 D [11]. The param-

eters of the AVTD curve in the cell lysate are determined by the genome conformational state, *i.e.* by hydrodynamic parameters of chromosomal DNA macromolecules which in their turn depend on the DNA nativity, DNA association with various proteins, the microenvironment, *etc.* The rotor's maximum rotation period (T_{max}) which in this method is the most sensitive parameter characterizing the genome conformational state of *E. coli* cells, was obtained from the AVTD curve. The measurement error of the rotor's rotation period was 2%.

Results

Irradiation of *E. coli* cells with doses of 10–50 Gy leads to changes of the AVTD curve of the cell lysate (Fig. 2, curve 2). The major cause of these changes is the considerable decrease of T_{max}. After post-irradiated cell incubation for 90–120 min, depending on the dose of irradiation, an almost complete recovery of the AVTD curve (Fig. 2, curve 3) took place. This means that during this period the GCS of the irradiated cells returned to the control level. It is in this sense that we use the term "repair" of the genome conformational state.

In preliminary experiments the X-irradiated cells were exposed to microwaves in the regime of frequency switching. This was brought about within the range of about 200 MHz during 30–90 min. Fig. 2 (curve 4) shows the AVTD curve after cell irradiation within the frequency band of 51.60–51.78 GHz at PD = 3 mW/cm² for 90 min.

It can be seen that microwaves in this range effectively suppress repair of the GCS. To assess the microwave effect on the repair process after X-irradiation, we used the following ratio:

$$\varkappa = \frac{\bar{T}_{\max XR+I} - \bar{T}_{\max eff}}{\bar{T}_{\max XR+I} - \bar{T}_{\max XR}}$$

where:

$\bar{T}_{\max XR}$ – the average maximum rotor's rotation period in the lysates of cells lysated immediately after X-irradiation;

$\bar{T}_{\max XR+I}$ – the average maximum rotor's rotation period in the lysates of cells lysated after X-irradiation and subsequent incubation (I);

$\bar{T}_{\max eff}$ – the average maximum rotor's rotation period in the lysates of cells subjected to EHF EMR during the radiation-induced repair. In two effective microwave ranges the $\varkappa$ dependencies on frequency were determined.

In these experiments, cells were irradiated with microwaves of a certain frequency for 5 to 15 min after X-irradiation. To assess the average value of the rotor's maximum rotation period ($\bar{T}_{max}$) in each of the experiments 3 AVTD measurements were made. Significance level was determined by the Student's t-test. The extent and results of a standard experiment are given in Table I.

Fig. 3 and Fig. 4 present the $\varkappa$ dependence in the ranges examined: 51.62–51.84 GHz (strain

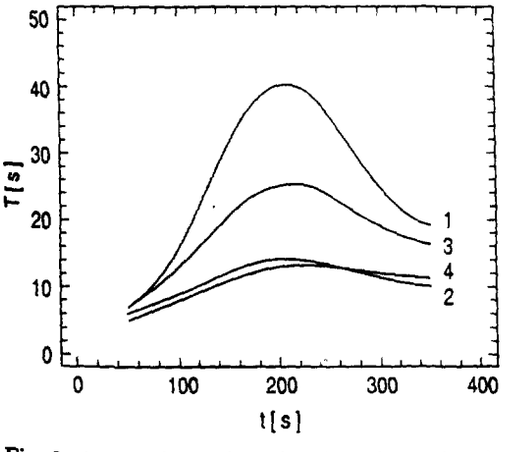

Fig. 2. Anomalous viscosity time dependencies of *E. coli* G62 cell lysates: 1 – control; 2 – X-irradiation (30 Gy); 3 – XR and incubation (90 min); 4 – XR and incubation under the influence of EHF EMR.

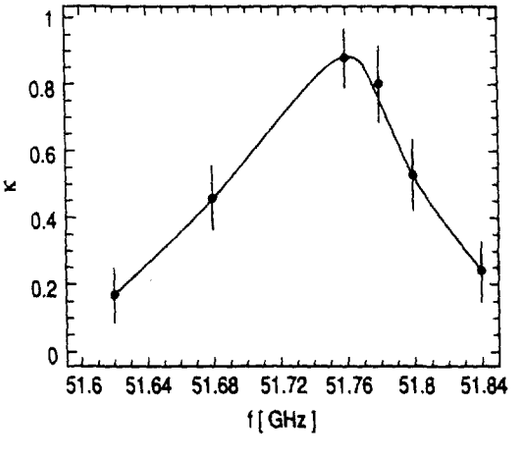

Fig. 3. Frequency dependence of EHF EMR effect on radiation-induced repair of the GCS of *E. coli* RM117 cells (20 Gy; 15 min, 3 mW/cm²).

Table I. Values of the maximum rotor's rotation period derived from AVTD curves obtained in lysates of *E. coli* AB1157 cells, lysated after X-irradiation (20 Gy), subsequent incubation or irradiation with EHF EMR (200 μW/cm²) in the course of incubation.

Type of effect	EMR frequency [GHz]	Duration of EHF EMR irradiation [min]	T_{max} [S]	$\bar{T}_{max} \pm SE^*$ [S]	Significance level as compared with XR + I
Control	–	–	51.1 35.1 47.8	44.8 ± 4.8	$p < 0.03$
XR	–	–	7.4 7.2 6.5	7.0 ± 0.3	$p < 0.0001$
XR + I	–	–	28.1 24.7 25.7	26.2 ± 1.0	–
	41.25	10	14.0 12.3 12.1	12.8 ± 0.6	$p < 0.0004$
XR + EMR + I	41.30	10	7.2 6.4 7.2	6.9 ± 0.3	$p < 0.0001$
	41.35	10	8.9 10.1 10.1	9.7 ± 0.4	$p < 0.0002$
	41.40	10	9.7 11.2 12.2	11.0 ± 0.7	$p < 0.0004$
	41.45	10	16.2 17.3 16.6	16.7 ± 0.3	$p < 0.001$
	41.50	10	15.2 16.2 15.6	15.6 ± 0.3	$p < 0.0006$

* Standard error.

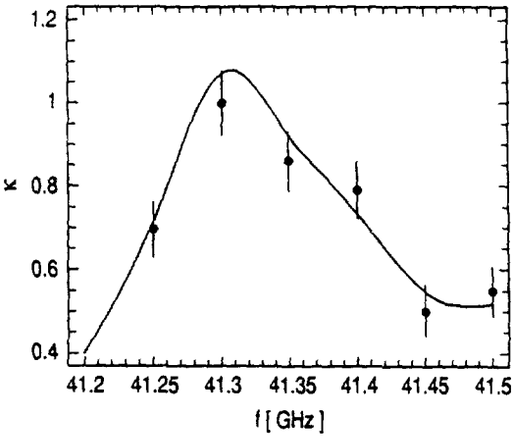

Fig. 4. Frequency dependence of EHF EMR effect on radiation-induced repair of the genome conformational state of *E. coli* AB1157 cells (20 Gy; 200 μW/cm², 10 min).

RM117) and 41.25–41.50 GHz (strain AB1157). It is clear that in both ranges this dependence has a resonance nature with a resonance half-width of the order of 100 MHz and resonance frequencies of 51.76 GHz and 41.32 GHz respectively. In the first instance the cell exposure to EHF EMR was carried out at PD = 3 mW/cm². The SAR value, estimated by acoustic and calorimetric methods, amounted to 17 mW/g and 22 mW/g respectively. Heating of the cell suspension, when irradiated, did not exceed 1 °C. The κ dependence on frequency within the range of 41.25–41.50 GHz was studied at PD = 200 μW/cm² with heating not exceeding 0.1 °C. It should be noted that heating of a cell suspension by 5 °C for 10 min right after the X-irradiation did not lead to suppression of repair

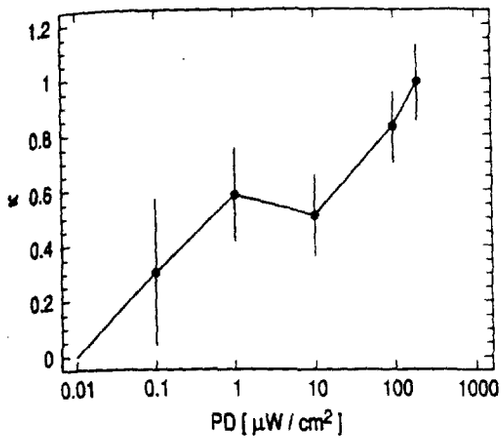

Fig. 5. Dependence of suppression effectiveness of radiation-induced GCS repair on microwave PD (strain AB1157, 20 Gy; 41.32 GHz, 5 min).

processes. We also studied the dependence of suppression of radiation-induced GCS repair on PD of the microwave exposure at the 41.32 GHz frequency. The power dependence of $\varkappa$ is shown in Fig. 5. Starting with a PD of 1 $\mu W/cm^2$, irradiation for 5 min significantly suppressed GCS repair.

As pointed out above, Fig. 3 shows a frequency dependence within the 51.62–51.84 GHz range for RM117 strain. But this microwave irradiation was effective in repair suppression for the other strains used: AB1157 and G62. Altogether 11 experiments were carried out, each revealing statistically significant suppression of repair processes by microwaves at frequencies of this resonance.

An EHF EMR effect on the genome conformational state was also discovered in the case of inverse sequence of cell exposure to microwaves and X-rays. Irradiation of cells with EHF EMR at the 51.78 GHz frequency (that is close to that of resonance) before X-irradiation prevented the process of radiation-induced repair (Table II).

Table II. Values of the maximum rotor's rotation period in cell lysates after a combined effect EHF EMR (3 mW/cm^2, 51.78 GHz, 30 min) and XR (30 Gy) on *E. coli* RM117 cells.

Type of effect	$\bar{T}_{max} \pm SE$ [S]	Significance level as compared with XR + I
Control	17.1 ± 0.9	$p < 0.04$
XR	6.9 ± 0.1	$p < 0.02$
XR + I	12.5 ± 1.4	–
EMR + XR + I	7.2 ± 0.2	$p < 0.003$

Discussion

It is generally accepted that biological membranes are receptors of chemical and electromagnetic signals. Can this premise alone explain those resonance bioeffects which can be seen when cells are subjected to low-intensity millimeter radiation? This resultant effect can change such important biological parameters as velocity of cell division [1, 2] or processes of gene expression [3, 5]. It would seem that the simplest answer to the question of the target of microwave resonance effect is that the target is the cell membrane whose properties determine frequencies of resonant interaction. Indeed, in a number of model studies microwave effects were detected that had been caused by a change in the ion membrane transport [13–15]. But the microwave "membrane" effects examined did not depend on the EMR frequency and therefore do not permit explanation of the resonance effect on the processes of cell development and gene expression. It appeared to us that a promising explanation of these observations could be supplied by the notion of the role of the genome conformational state in forming cell's resonance response to the millimeter wave exposure. In other words, we assumed that parameters of the GCS, *i.e.* space-topological organization of chromosomal DNA, determine resonance frequencies. In such an event the GCS would be sensitive to the effect of millimeter waves of certain frequencies.

In order to provide support for this supposition we used the method of anomalous viscosity time dependencies in cell lysates, which has a high sensitivity to the GCS change [6]. Changes in the AVTD can be detected even with an X-ray dose of 10 cGy when less than one single-strand DNA break is induced per *E. coli* genome. This result already made it possible to assume that the AVTD method is sensitive not only to damages of the sugar-phosphate bonds of the DNA secondary structure. The AVTD sensitivity to other changes of the genome conformation, particularly those caused by DNA-protein bonds, was confirmed by the experiments we carried out [16]. The results obtained in the course of our work indicate that repair of the genome conformational state of bacterial cells after ionizing irradiation is highly sensitive to the resonance effect of millimeter waves.

The microwave effect discovered cannot be explained by trivial heating. This was borne out by

many of the results obtained. First, there were effective PD of about 1 $\mu W/cm^2$, while SAR amounted to 10 $\mu W/g$ is not enough for a noticeable heating of the irradiated suspension over 5–15 min. Second, heating of the cell suspension by 5 °C for 10 min during the postradiative incubation has no influence on the restoration. Finally, the PD averaged over the irradiated surface did not depend on the frequency within the limits of the observed resonances (± 200 MHz).

There is hardly any doubt that destabilization of repair and probably other protein complexes with DNA is the central event of the molecular-biological mechanism preventing the GCS repair. Surprisingly, this effect may be obtained even if cells are subjected to EMR with resonance frequency before X-irradiation. This result means that a cell, irrespective of whether or not it was X-irradiated, retains the microwave resonance effect for a certain period. It is especially important to stress that this memory is realized at the level of the genome conformational state. This inference is supported by the fact that after a 5–10 min EMR effect on X-irradiated cells, the prevention of GCS repair persists for at least an hour and a half of the subsequent incubation.

The discovered frequency dependence of the effect, especially the half-width of resonances (100 MHz), is similar in character to that which had been obtained when studying the gene expression of repressed λ-prophage operon in lysogenic *E. coli* cells [3, 5]. In our view, this coincidence is one more argument in favour of the supposition of the role of the genome conformational state in the resonance response of bacterial cells to a millimeter wave effect.

In general, a chain of events seems to be involved in this interaction. At the first stage, microwaves interact with cell membranes. It is likely that the signal in the membrane intensifies and is received in the DNA through the point (points) where DNA is attached to the membrane. We believe that there are parameters of DNA or its selected sites, including those bound with proteins, that determine the resonance frequencies of electromagnetic waves capable of influencing the genome conformational state through the membrane.

One cannot exclude the possibility that the primary targets of millimeter wave action are proteins, which take part in maintaining the structural and functional integrity of chromosome DNA [18, 19]. Then changes in the GCS registered by the AVTD method will be defined by the influence of EHF EMR on the function of these proteins. By affecting the GCS through the processes of molecular interaction the microwaves may give rise to changes of DNA secondary structure, changes in elementary genetic processes: transcription, replication, repair and recombination. Consequently, it is possible to record the final biological effect at the cell level: modification of gene expression down to derepression of operons [2–5], changes in the velocity of DNA synthesis and in cell division [1, 17].

It is worth noting that cells of all the *E. coli* strains used (AB1157, RM117, G62) were sensitive to EMR of the 51.62–51.84 GHz frequency band. The first two of these strains are isogenic by known markers. As to the third strain, it differs from the previous ones by a number of markers. For instance, G62 cells have no mutations in the gene of acetylornithine deacetylase or other genes whose products take part in the biosynthesis of arginine and therefore are not auxotrophic on this amino acid. It is possible that structural genes whose mutations determine differences in the strains used have no relationship with a mechanism of resonance interaction. But it appears likely to us that resonance frequencies are determined by regulatory nucleotide sequences and their mutual position within cellular DNA.

The results obtained in this work are in accordance with the physical models predicting the existence in living systems of discrete resonance states corresponding to the millimeter band of an electromagnetic field [18, 19].

A further experimental confirmation of the genome's role in giving rise to these discrete states and the existence of selection rules on helicity for transitions between them will be made public at a later date.

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Short communication

Some biophysical aspects of the genetic effect of low-intensity millimeter waves *

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Considering the genetic effect of physical and chemical agents, two main groups of effects can be picked out. The first group includes the main mutagenic effects, resulting in heritable changes in the genetic material (genome, chromosomes, genes). The second group consists of epigenetic effects of the agents, i.e. effects on the interaction of genes and their products in the process of gene expression. The epigenetic effects of extremely high-frequency electromagnetic radiation (EHF EMR) were found to form the base of one of the paradoxes in modern biophysics, resembling the well-known radiobiological paradox in nature. An effect specific to EHF EMR (millimeter waves) is the effectiveness of low non-thermal intensities, firstly, and, secondly, the dependence of the effects on EMR frequency and polarization. The most striking example of the effect of resonance epigenetic millimeter waves was detected by Webb [1] while investigating λ-prophage induction in lysogenic cells of *E. coli*. As a matter of fact, the prophage induction was found to be the result of gene switching from a lysogenic to a lytic path of development. Interestingly, Webb's results could not be reproduced by other authors in a number of cases (Table 1). It should be noted, however, that not all methodological conditions described by Webb [1], were met in the investigations with negative results. In our experiments, EMR induced resonantly a release of prophage from wild-type *E. coli* cells [11]. However, the value of induction was shown to be much lower than that in the study of Webb [1]. The induction effectiveness in a Rec A mutant strain, in which neither ionizing radiation nor

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TABLE 1

Epigenetic effect of the EHF EMR on *E. coli* cells

Year (reference)	Object	Parameters of exposure f and PD	Observed effects, its value $K = N_{eff} / N_{cont}$
1973 (2)	*E. coli* C 600 (colE$_1$)	42.25 GHz 46.15 GHz 51.75 GHz 0.001–1 mW/cm^2	induction of colicine synthesis. $K_{max} = 3$
1978 (3)	*E. coli* W3110 (colE$_1$)	45.5–46.2 GHz	colicine synthesis. $K_{max} = 8$, $f_{res} = 45.90$ GHz
1979 (4)	*E. coli* W 3110 (colE$_1$) BR-475 (λ, lac$^-$)		no colicine synthesis, no prophage induction
1979 (5)	*E. coli* W 3110 (colE$_1$)	51.3–52.2 GHz 0.5 mW/cm^2	colicine $K_{max} = 2$, $f_{res} = 51.70$ GHz
1979 (1)	*E. coli* K12 (λ)	70.3–70.5 GHz 0.2–0.5 mW/cm^2	prophage induction, $K_{max} = 10^5$, $f_{res} = 70.4$ GHz
1980 (6)	*E. coli* K12(λ)	40–60 GHz 0.1–0.2 mW/cm^2	prophage induction, $K_{max} = 1.3$, $f_{res} = 41.268$ GHz
1983 (7)	*E. coli* W 3110 (colE$_1$)	33–75 GHz 0.5–5 mW/cm^2	no colicine synthesis
1983 (8)	*E. coli*		no colicine synthesis
1983 (9)	*E. coli* C 600 (λ)	41–48 GHz 65–75 GHz	no prophage induction
1984 (10)	*E. coli*		no prophage induction
1990 (11)	*E. coli* K12 N 99 (λ) N 99 (λ, recA$^-$)	70.30–70.60 GHz 0.1–0.5 mW/cm^2	prophage induction, $K_{max} = 20$, $K_{max} = 10^3$, $f_{res} = 70.37$ GHz

ultraviolet can induce prophage, was higher by 2 orders of magnitude. The Rec A protein performs a key function in the mechanism of prophage λ gene switching under the action of DNA-damaging agents. The resonance transition of prophage from lysogenic to lytic development in the mutant cells under study shows that the mechanisms of prophage gene expression under the effect of mm waves and DNA-damaging agents have to be essentially different. This result also supports

TABLE 2

Examples of the frequency-dependent epigenetic effect of the EHF EMR on higher eukaryotes

Year (reference)	Object	Parameters of exposure f and PD	Observed effects
1983 (12)	*Acricotopus lucidus*	64.1–69.1 GHz (67.2 and 68.2 GHz) 5 mW/cm^2	regression of the BR2 puff of polytene chromosomes. reduction of RNA synthesis and protein secretion: $P < 0.05$
1983 (13)	*Drosophila melanogaster*	41.67 GHz 5 mW/cm^2	significant induction of somatic mosaicism
1989 (14)	*Drosophila melanogaster*	46.00–46.50 GHz (46.35 GHz) 0.1 mW/cm^2	increased level of morphoses: $P < 0.01$
1989 (15)	*Crepis capillaris*	41.01–42.96 GHz 0.02–2.5 mW/cm^2	seven-fold increase in centromeric decondensation of A chromosomes: $P < 0.05$

the suggestion about the direct influence of EHF EMR on the conformational state of DNA–λ repressor complex.

There is reason to suppose the frequency-dependent EHF EMR effect is universal and independent of the evolutionary complexity of an organism. Examples of this effect in prokaryotes are well known (Table 1). Table 2 gives the data on the epigenetic effect of mm waves on high eukaryotes. It has been established [14], that the earlier in ontogenesis an effect occurs, the higher is the ability of EMR to induce the formation of morphoses (i.e. individuals with morphological developmental defects). The switching of individual genes and their clusters was found to occur most frequently at early stages. An illustrative example of this phenomenon is the self-coordinated expression of the fushi tarazu gene [16] early in the development of the embryo of *D. melanogaster*. Probably, an internal electromagnetic field synchronizes the processes of gene expression. Assuming the existence of a stationary wave causing the self-coordinated expression of the fushi tarazu gene and taking into account the dielectric permeability of tissue [17], one can assess the wavelength (λ_0) in vacuum and the characteristic EMR frequency:

$$\lambda_0 = \lambda_{ts}\sqrt{\frac{\epsilon}{2}\left(1+\frac{\epsilon}{\epsilon_0}\right)^2 + 1)} \approx 0.1 \text{ mm}$$

This frequency (3000 GHz) lies in the submillimeter microwave range (hyperhigh frequencies). The presence of characteristic frequencies in a wide range of EMR was shown experimentally in studies of the Raman spectra of *E. coli* synchronous cells [18].

Specifically, the mechanism of the epigenetic effect of mm waves might, at least in a number of cases, be explained by stabilization of weak coherent interactions of DNA with structural and functional proteins. Many DNA–enzymatic complexes (replication or repair complexes, and so on) are connected with the nuclear matrix (in eukaryotes) and membrane. Exactly by means of these connexions, interregulation of membranes and DNA can occur as a result of a response to resonant EHF EMR. The central role of the genome in the process was demonstrated in a study of the effect of mm waves on the conformational state of the genome in *E. coli* cells [19]. The appearance and repair of conformational changes in chromosomal DNA were investigated by the original method of viscosity anomalous time dependence (VATD) in lysates of irradiated cells. This method provides the possibility to study changes in the conformational state of a cell's genome in the case of extremely weak effects which cause no direct damage in the DNA [20]. EMR in the ranges of 70.3–70.6, 51.62–51.84 and 41.25–41.50 GHz was shown [19,21] to cause changes in VATD in lysates of irradiated cells with an effectiveness of the same order as X-radiation at LD_{50} dose. However, in the first case, no cell death occurred. The results obtained mean that EHF EMR changed resonantly the conformational state of the genome, i.e. changed its stereochemical code by influencing the self-coordination of DNA–protein interactions. In this case one can expect systemic response of cells, namely both a change in gene expression, detected really by λ-prophage induction [11,19], and changes in replication processes and in cell-division rate [19].

An important result was obtained when investigating the effect of EHF EMR with different polarizations on the conformational state of the genome of *E. coli* cells. At one of the resonance frequencies investigated (51.62–51.84 GHz), right-circular polarized mm waves were found to be maximally effective in suppressing X-ray-induced reparation of the genome's conformational state, whereas linear polarization appeared to have a mediocre effect, and left-circular EHF radiation had practically no effect at all. At one of the other resonance frequencies investigated (41.25–41.50 GHz), the reverse picture was observed, i.e. the left polarization was effective, whereas the right-circular polarized radiation had no effect on the reparation of the genome's conformational state. The results obtained seem to indicate that the helix rules of selection operate during discrete transitions in living systems.

The most important aspects of this problem should be emphasized. Firstly, transitions between discrete states of the system, as a whole, can be accomplished in the EHF range. Secondly, the central link in the resonant response of cells to low intensity mm waves is the conformational state of the genome which determines the stereochemical code. As to the results obtained by us, they might open some perspective for a common understanding of such fundamental properties of biological systems as the chirality of biomolecules [22], that is, from the point of view of physics, non-conservation of evenness; the presence of discrete levels and selection rules for the transitions between them.

As regards the mutational aspect of the genetic EHF EMR effect, it should be

noted that the problem has become especially important, since experimental data have emerged on the DNA damaging effect of microwaves in vitro [23]. In this case, preparations of plasmid DNA were exposed to microwaves with a resonant frequency of 2.55 GHz as calculated from electroacoustic theory [24]. It was found, that non-thermal levels of microwave exposure resulted with high efficiency in a

TABLE 3

Direct and indirect indications of non-mutagenic action of the EHF EMR in vivo

Years (reference)	Object	Parameters of exposure *f* and PD	Observed effects
1976 (25)	excision and recombination repair defective mutants of *E. coli* K12	70–75 GHz < 60 mW/cm^2 70.5 GHz 73.0 GHz	affected growth rate without cell death (no DNA damages)
1976 (25)	*E. coli* K12 tryp	70–75 GHz < 60 mW/cm^2 70.5 GHz 73.0 GHz	no back mutations tryp$^-$ → tryp$^+$
1976 (25)	*S. cerevisiae*	70–75 GHz < 60 mW/cm^2 70.5 GHz 73.0 GHz	qno back mutations his$^-$ → his$^+$; no cytoplasmic mutations
1976 (25)	*S. cerevisiae* defective in repair		no cell death (no DNA damages)
1979 (26)	haploid strains of *S. cerevisiae* 123 and 211-laM; diploid strain D_5-Zimmerman	70.5–73 GHz 6–60 mW/cm^2	no mitotic recombination and no cytoplasmatic mutations
1981 (27)	*S. cerevisiae*	41.67 GHz 0.5 mW/cm^2	changed development of populations without mutations in the gene of ANP-cocarboxylase
1983 (13)	*D. melanogaster*	41.67 GHz 5 mW/cm^2	induction of somatic mosaicism without dominant lethal mutations
1987 (28)	*E. coli* G 53 (P 386) IM (pBR325)	1 mW/cm^2	changed level of drug resistance and division rate without changes in plasmid DNA
1989 (15)	*C. capillaris*	37.46–78.40 GHz 0.02–10 mW/cm^2	frequency-dependent de-condensation of chromatin without abberations of chromosomes
1989 (19)	strains of *E. coli* (K12) defective in repair of recA$^-$ (AB2463) and polA$^-$ (P3478)	70.3–70.6 GHz 0.5 mW/cm^2 41.264 GHz 3 mW/cm^2	changed conformational state of genome without cell death (no DNA damages)

transition of the plasmid from its supercoiled form into open circular and linear forms. This means that microwave radiation can produce breaks in single- and double-stranded DNA. The estimated length of nucleotide sequences resonating by that pattern in the EHF range is 50 bp. Segments of such length consisting of inverted repeats and having the ability to form hairpins and other structures, can be found within cellular DNA.

We have analyzed the available data, allowing us to judge, directly or indirectly, the possible mutagenic effect of EHF EMR (Table 3). We found that, in all cases with a frequency-dependent effect on cell growth rate, developmental processes and so on, mm waves had no mutagenic effect. In disagreement with these results are data reported by Zalyubovskaya [29], who recorded mutations in the second generation of *Drosophila*. Unfortunately, an analysis of the above-mentioned paper is difficult because no description is given of the experimental conditions such as power density, details of the mutation assay and so on. On the other hand,

TABLE 4

Heritable modifications induced by the EHF EMR action

Year (reference)	Object	Parameters of exposure *f* and PD	Observed effects
1977 (30)	*Aspergillus oryzal*	46.88–46.15 GHz	increased fibrinolytic activity
1981 (31)	*Endomicopsis filulger*	50 GHz 48.39 GHz	50% increased aminolytic activity (1 year)
1981 (32)	*S. cerevisiae*	41.67 GHz 0.5 mW/cm^2	changed development of population (300 generations)
1983 (33)	Hyphal fungus	41.67 GHz 0.5 mW/cm^2	changed development of population, shifted maximum of intensity of hydrolytic enzyme synthesis (three-fold re-inoculation, "many generations")
1987 (34)	*S. carlsbergens, s-lvovskaya strain*	49.59 GHz	increased capability to ferment maltose (100 generations)
1987 (28)	*E. coli* G 62 (1–19) G 53 (P 386) IM (pBR 325)	48.46 GHz 48.15 GHz 48.07 GHz 47.10 GHz 46.66 GHz 46.30 GHz 46.22 GHz 46.15 GHz 46.08 GHz 1 mW/cm^2	changed level of resistance to antibiotics

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Considering the genetic effect of physical and chemical agents, two main groups of effects can be picked out. The first group includes the main mutagenic effects, resulting in heritable changes in the genetic material (genome, chromosomes, genes). The second group consists of epigenetic effects of the agents, i.e. effects on the interaction of genes and their products in the process of gene expression. The epigenetic effects of extremely high-frequency electromagnetic radiation (EHF EMR) were found to form the base of one of the paradoxes in modern biophysics, resembling the well-known radiobiological paradox in nature. An effect specific to EHF EMR (millimeter waves) is the effectiveness of low non-thermal intensities, firstly, and, secondly, the dependence of the effects on EMR frequency and polarization. The most striking example of the effect of resonance epigenetic millimeter waves was detected by Webb [1] while investigating λ-prophage induction in lysogenic cells of *E. coli*. As a matter of fact, the prophage induction was found to be the result of gene switching from a lysogenic to a lytic path of development. Interestingly, Webb's results could not be reproduced by other authors in a number of cases (Table 1). It should be noted, however, that not all methodological conditions described by Webb [1], were met in the investigations with negative results. In our experiments, EMR induced resonantly a release of prophage from wild-type *E. coli* cells [11]. However, the value of induction was shown to be much lower than that in the study of Webb [1]. The induction effectiveness in a Rec A mutant strain, in which neither ionizing radiation nor

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TABLE 1

Epigenetic effect of the EHF EMR on *E. coli* cells

Year (reference)	Object	Parameters of exposure f and PD	Observed effects, its value $K = N_{eff}/N_{cont}$
1973 (2)	*E. coli* C 600 (colE$_1$)	42.25 GHz 46.15 GHz 51.75 GHz 0.001–1 mW/cm^2	induction of colicine synthesis. $K_{max} = 3$
1978 (3)	*E. coli* W$_n$3110 (colE$_1$)	45.5–46.2 GHz	colicine synthesis. $K_{max} = 8$, $f_{res} = 45.90$ GHz
1979 (4)	*E. coli* W 3110 (colE$_1$) BR-475 (λ, lac$^+$)		no colicine synthesis. no prophage induction
1979 (5)	*E. coli* W 3110 (colE$_1$)	51.3–52.2 GHz 0.5 mW/cm^2	colicine $K_{max} = 2$, $f_{res} = 51.70$ GHz
1979 (1)	*E. coli* K12 (λ)	70.3–70.5 GHz 0.2–0.5 mW/cm^2	prophage induction. $K_{max} = 10^5$, $f_{res} = 70.4$ GHz
1980 (6)	*E. coli* K12(λ)	40–60 GHz 0.1–0.2 mW/cm^2	prophage induction. $K_{max} = 1.3$, $f_{res} = 41.268$ GHz
1983 (7)	*E. coli* W 3110 (colE$_1$)	33–75 GHz 0.5–5 mW/cm^2	no colicine synthesis
1983 (8)	*E. coli*		no colicine synthesis
1983 (9)	*E. coli* C 600 (λ)	41–48 GHz 65–75 GHz	no prophage induction
1984 (10)	*E. coli*		no prophage induction
1990 (11)	*E. coli* K12 N 99 (λ) N 99 (λ, recA$^-$)	70.30–70.60 GHz 0.1–0.5 mW/cm^2	prophage induction. $K_{max} = 20$, $K_{max} = 10^3$, $f_{res} = 70.37$ GHz

ultraviolet can induce prophage, was higher by 2 orders of magnitude. The Rec A protein performs a key function in the mechanism of prophage λ gene switching under the action of DNA-damaging agents. The resonance transition of prophage from lysogenic to lytic development in the mutant cells under study shows that the mechanisms of prophage gene expression under the effect of mm waves and DNA-damaging agents have to be essentially different. This result also supports

there are many available publications on the ability of EHF EMR to produce heritable biochemical, morphological and other changes in microorganisms (Table 4). As a rule, these changes result from a prolonged (many hours) EMR effect at resonant frequency and apply to the development of population, enzymatic activity, drug resistance. Analysis of experimental results has demonstrated that mm waves can be a factor in the variability of prolonged modification. Consequently, there are no grounds to connect the observed modification processes with mutagenesis.

Thus, one can conclude, that at present there is no experimental evidence for the mutagenicity of millimeter waves at non-thermal level. At first sight, with the presence of epigenetic effects of resonant EHF EMR, this seems to be paradoxical. However, taking into account the data on the specificity of the biological action of EHF EMR, we arrive at the conclusion that microwaves play a fundamental role in the maintenance of homeostasis within the limits of the reaction norm, determined genetically for every species. This conclusion answers the question raised on the mutagenicity of mm waves and allows us to assume, for the effect of EMR in a given diapason, that the mutagenicity cannot exceed the maximum possible control level.

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Specific Absorption Rate in Rats Exposed to 2,450-MHz Microwaves Under Seven Exposure Conditions

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Both positive and negative biological effects of microwaves on drug actions in rats exposed to 1-mW/cm^2, 2,450-MHz microwaves have been reported by several investigators. We conducted dosimetry studies for seven different exposure conditions to determine whether these different results could be due to the rats having been exposed differently. They included anterior and posterior exposures in a circular waveguide, near field, far field with E- or H-field parallel to the long axis of the body and dorsal exposure in a miniature anechoic chamber with E- or H-field parallel to the long axis of the body. The average specific absorption rates (SARs) in the head, tail, and body of the exposed rats were measured by means of a calorimetry system. The local SARs at eight locations in the brain were determined by temperature measurement with Vitek probes. Intensive coupling of energy to the tail when it was exposed parallel to the E-field was shown by thermography. For the same average incident power density, the average SARs in the heads of rats were about two times higher in the circular waveguide than for other exposures. The local SARs in the brain varied for different exposure conditions. Statistical comparisons of SARs under the different exposure conditions are presented.

Key words: SAR, rat, head, tail, brain sites, 2,450 MHz, calorimetry

INTRODUCTION

During the last few years, there have been a few studies on the effects of microwave exposure on drug actions. Both positive and negative results for rats exposed to 1-mW/cm^2, 2,450-MHz microwaves have been reported by different investigators [Thomas et al, 1977, 1979a, b, 1980; Thomas and Maitland, 1979; Hamilton et al, 1981; Johnson et al, 1981; Sessions, 1981; Lai et al, 1983, 1984; DeWitt and D'Andrea, 1984]. The different results could be due to the various exposure conditions used for the studies.

It is known that power absorption in tissue varies with many factors, including frequency, polarization, and power density of the incident electromagnetic fields, as well as the size and orientation of the animal. In addition, the coupling of microwave energy to the animal can differ depending on exposure conditions: near field, far field, or guided electromagnetic radiation.

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In order to determine the dose-response relationship of biological effects due to microwave exposure of animals, it is necessary to measure the specific absorption rate (SAR) in the tissues where microwaves exert their effects. Since most microwave-drug interaction studies involved behavioral effects, it is important to determine whether the SARs in the brains of the exposed animals used in the reported studies were the same. The work reported in this paper was directed toward establishing this comparison. We determined the average SAR in the head and whole body, and also the local SARs at eight locations in the brains of rats exposed under the seven exposure conditions used by investigators conducting microwave-drug interaction studies. In addition, we determined the SAR in the tail of the animals because of the suspected high energy absorption at that location. Though all previous biological studies have involved pulsed-microwave exposure, dosimetry studies can be based on the use of continuous-wave (CW) sources. The higher power available from such sources will produce sufficient temperature rise in the exposed tissue so that thermometry methods for determining SAR may be used. The average SAR, electric field strength, current density, and specific absorption (SA) per pulse in exposed tissue for low-power-density pulsed exposures, can easily be calculated from the SAR values obtained from high-power CW exposures.

METHODS AND MATERIALS

Subjects

Male Sprague-Dawley rats of the same body-weight range (250–350 g) as that for animals used in the various drug studies were obtained from Tyler Laboratories (Bellevue, WA).

Exposure Conditions

The six exposure conditions reported by investigators of microwave-drug interactions on animals are as follows:

1. 2,450-MHz, near-field exposure of restrained rats with the major body axis parallel to H-field [Thomas et al, 1977, 1979a, b]
2. 2,800-MHz, near-field exposure of restrained rats with major body axis parallel to H-field [Schrot et al, 1980]
3. 3,000-MHz, far-field exposure of restrained rats with the major body axis parallel to H-field [DeWitt and D'Andrea, 1984]
4. 2,880-MHz, far-field exposure of restrained rats with the major body axis parallel to E-field [Lovely, et al, 1981]
5. 2,450-MHz, circular polarized-waveguide exposure of freely moving rats [Hamilton et al, 1981; Johnson et al, 1981; Lai et al, 1983, 1984; Sessions, 1981]
6. 2,450-MHz, miniature-anechoic-chamber exposure of freely moving rats [Lai et al, unpublished data]

It would be ideal if the measurements could be done exactly under the conditions reported by the original investigators. However, a high-power, 2,800–3,000-MHz generator with several kilowatts of average output power was not available. A more practical choice was to expose the animals to 2,450 MHz instead of 2,800–3,000 MHz, since the difference in whole-body average SAR for a medium-sized rat between the different frequencies is expected to be less than 5% [Durney et al, 1978].

The rats were exposed to 2,450-MHz microwaves using three different systems: a circular waveguide system [Guy et al, 1979], a large RF-shielded room, and a miniature anechoic chamber [Guy, 1979]. Since the rats could move freely in the waveguide and the miniature chamber systems, anterior and posterior exposures of the animals in the waveguide and exposures with the major body axis parallel and perpendicular to the E-field in the chamber were conducted. Thus SAR information for seven different exposure conditions as described below were obtained.

1. Anterior exposure in circular waveguide system. A rat carcass was exposed in the center of the circular waveguide system with its head toward the transmitting transducer (Fig. 1). The waveguide system has been described in detail by Guy et al [1979]. The tail of the rat was curved along side of the body since the cage would not accommodate the rat with its tail fully extended.

2. Posterior exposure in circular waveguide system. The same procedure was followed as above, except that the rat was exposed posteriorly, ie, head pointing away from the transmitting transducer.

3. Near-field exposure, with H-field parallel to body. Dr. John Thomas, of the Naval Medical Research Institute, provided us with one of the cylindrical rat holders that he had used in his studies. The holder consisted of two concentric 15-cm-long Plexiglas cylinders, one of which (6.3 cm in diameter, 0.38 cm thick) was pushed into the other (7.6 cm in diameter, 0.63 cm thick). In our experiments, the holder and enclosed rat were placed horizontally on a Styrofoam stand and exposed to vertically polarized, 2,450-MHz microwaves in a 12 × 24 × 12-ft, radiofrequency anechoic chamber (Fig. 2). The rat's tail was allowed to hang down parallel to the E-

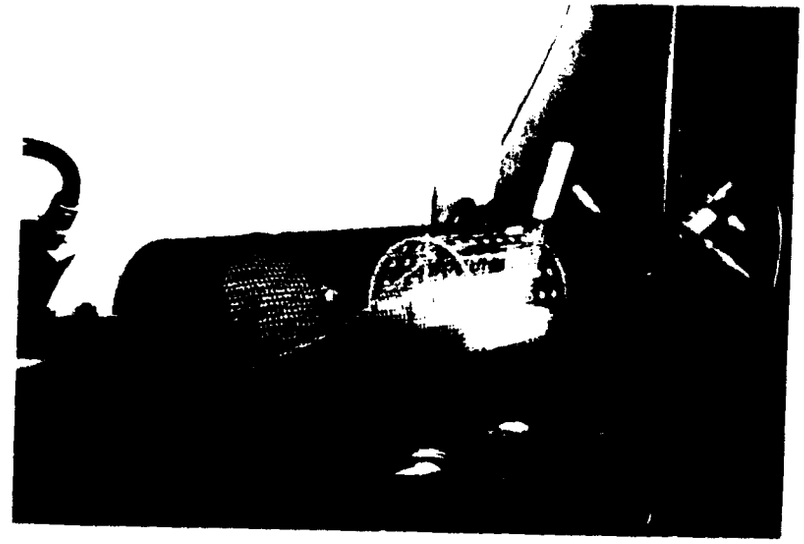

Fig. 1. Exposure (anterior) of rat in circular waveguide.